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Study of Biocomposite Films Based on Cassava Starch and Microcrystalline Cellulose Derived from Cassava Pulp for Potential Medical Packaging Applications

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Abstract: This study aimed to develop biocomposite films based on cassava starch and microcrystalline cellulose (MCC) derived from cassava pulp for potential medical packaging applications. MCC was extracted from cassava pulp, and its structure and chemical composition, crystallinity, and thermal properties were characterized. The MCC showed a yield of 14.92% and crystallinity of 46.91%. Different MCC contents (1%, 3%, and 5% *w*/*w* of starch) were incorporated into cassava starch films. The effects of MCC contents on film properties, including morphology, thickness, mechanical strength, chemical interactions, moisture content, surface wettability, and water activity index, were studied. The effects of UV-C sterilization on the disinfection of starch/MCC on film properties were determined. Results showed that all starch/MCC films exhibited good transparency and thickness ranging from 127 to 144 µm. As MCC content increased from 1 to 5%, Young's modulus and tensile strength of the films improved significantly from 112.12 to 488.89 MPa and 3.21 to 11.18 MPa, respectively, while elongation at break decreased from 44.74 to 4.15%. Incorporating MCC also reduced film surface wettability, with the water contact angle increasing from 69.17° to 102.82°. The starch/3%MCC holds promise as a biocomposite film for medical packaging applications, offering advantages in terms of good transparency, mechanical properties, and surface hydrophobicity. Furthermore, the absence of microbial growth in the sterilized gauze pad with sealing in the sterilized starch/3%MCC film confirms that the UV-C sterilization, 30 min for each side at 254 nm effectively eliminated any microorganisms present on the starch/3%MCC film without damaging the film properties. This finding highlights a reliable approach to ensuring the sterility of starch/MCC films for medical packaging applications.

Keywords: biocomposite films; cassava starch; microcrystalline cellulose; cassava pulp; medical packaging

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1. Introduction

Petroleum-based plastic packaging is commonly used for protecting and preserving food, cosmetic, pharmaceutical, medical, and chemical products [1]. However, the majority of synthetic plastics have low biodegradability due to the characteristics of the monomers and the covalent bonds between them, and they cannot be degraded by typical enzymatic, chemical, or physical processes [2]. As a result, burying or burning plastic garbage is a typical way of disposal, which pollutes the air, water, and land and endangers both human health and the ecosystem [3]. Nowadays, people are becoming more ecologically concerned,

and they are looking for new environmentally friendly materials to replace products obtained from fossil fuels. This issue can be solved by replacing non-biodegradable materials with biodegradable alternatives such as biopolymers, which are both ecologically friendly and harmless to health.

Many studies have been investigated using natural and biodegradable polymers for use in developing packaging films [4]. Starch is regarded as a highly compelling choice among biopolymers for developing packaging films due to its biodegradability, eco-friendliness, affordability, transparency, renewability, and ease of processing [5]. Unfortunately, starch film has poor mechanical properties. Many research studies solved these drawbacks by incorporating other components, such as chitosan, carboxymethylcellulose, and clays, into starch films [6]. Microcrystalline cellulose (MCC) is also an interesting reinforcing agent in the improvement in mechanical properties of cassava starch films because of excellent biodegradability, high strength, and low cost [7]. MCC is made from plants like cassava pulp, pineapple leaf, wood, sugarcane bagasse, bamboo, etc. [6]. Several investigations have indicated that adding MCC effectively increases the mechanical properties of starch-based composite films [8–10].

Until today, even though starch/MCC film fabrication has been reported, most of them have only focused on food packaging applications [10–12]. Numerous researchers confirmed that MCC enhanced the mechanical properties of starch films for food packaging applications. Othman et al. (2019) studied the mechanical properties of MCC-reinforced cassava starch films. In this work, MCC at different concentrations (0, 1, 3, 5, and 10% *w*/*w* of starch) was incorporated into cassava starch film. It was also found that by increasing the MCC content from 1% to 3% (*w*/*w* of starch), tensile strength and Young's modulus of the film significantly increased. When the MCC content was further increased to 5% and 10% (*w*/*w* of starch), tensile strength and Young's modulus dropped dramatically [10]. The reduction in mechanical strength occurred due to the formation of MCC agglomerates in starch films. Debnath et al. (2022) studied the effect of MCC derived from elephant grass on the mechanical properties of corn starch films. They found that by incorporating MCC from 1% to 5% (*w*/*w* of starch), the tensile strength and Young's modulus of the film increased from 6.04 MPa to 22.33 MPa and from 136.04 MPa to 493.29 MPa, respectively, whereas their elongation at break decreased from 29.35% to 3.49% [11]. Merci et al. (2019) found that the tensile strength of the cassava starch film was significantly increased when reinforced with MCC from soybean hulls. In addition, when increasing the MCC content from 2.5% to 5% (*w*/*w* of starch), the tensile strength of the starch film increased from 0.96 to 1.11 MPa while their elongation at break decreased from 76.10% to 11.36% [12].

Not only food packaging but medical packaging is also one of the large waste from plastic, which also needs to be replaced by biodegradable materials [13]. Certainly, the issue of medical packaging waste is significant and warrants attention, especially considering the environmental impact of plastic waste. Transitioning from traditional plastic medical packaging to biodegradable alternatives can help mitigate the environmental impact of plastic waste, reduce landfill usage, align with regulatory requirements and sustainability goals, and enhance public perception of healthcare organizations' environmental stewardship efforts. It represents a proactive step towards creating a more sustainable and environmentally responsible healthcare industry. Currently, there are no reports on biocomposite film for medical packaging applications due to the difficulties in the sterilization process, as it is sensitive to temperature and moisture [14]. Therefore, the development of biocomposite films and suitable sterilization methods remains a challenging task.

Sterilization stands as a pivotal process in the manufacturing of medical packaging. It ensures that packaging materials and components are devoid of harmful microorganisms, maintaining their sterility until they are employed [15]. The existing literature extensively documents that traditional sterilization methods like steam, gamma irradiation, and ethylene oxide can lead to damage, alterations, or the release of toxic substances, primarily because of the thermal and hydrolytic sensitivity inherent in biodegradable materials [14]. UV-C sterilization can indeed be considered an alternative method for sterilizing film

packaging. Generally, UV-C sterilization is a method used to sterilize medical devices and pharmaceutical products that are sensitive to temperature and moisture [16]. UV-C sterilization proves most effective at a wavelength of 254 nm, closely aligning with the peak absorption point of DNA. When bacteria or pathogens encounter UV-C radiation, their cells and genetic material sustain damage, disrupting their ability to replicate. UV-C radiation poses detrimental effects on a wide range of microorganisms, encompassing bacteria, bacterial spores, molds, mold spores, viruses, algae, and yeast [17]. UV-C sterilization is effective for air and surface disinfectants, but it may have limited penetration depth and may not be suitable for densely packed or opaque materials [18]. Katara et al. (2008) report that UV-C can have efficient inactivation of bacteria with an exposure time of 30 min [19]. However, the longer UV-C exposure time (3 h) significantly reduced the mechanical properties of sterilized materials [20].

In this study, biocomposite films based on cassava starch and MCC extracted from cassava pulp were developed for medical packaging applications. MCC extracted from cassava pulp has received considerable interest in the development of biocomposite films because of its distinctive mechanical characteristics, non-toxic and biodegradability [21,22]. Cassava pulp has been recognized for its significant cellulose content, reaching up to 20%, making it a suitable source for producing MCC [22]. It is obtained from the residual material of the cassava plant, one of the most important harvests in terms of annual production. However, its waste, around 7.3 million tons/year in Thailand, is rather significant, which can contribute to environmental issues [23]. The extraction of cassava pulp is one of the solutions to achieve effective use of natural resources and sustainable management, and cassava pulp became a rich source for the production of MCC without shortage. Although there have been documented successes in extracting MCC from cassava pulp, the exploration of its application in starch film reinforcement remains relatively unexplored [24,25]. Specifically, there is a noticeable dearth of research concerning the physical and mechanical attributes of starch films reinforced with MCC derived from cassava pulp.

In our work, MCC at different contents were incorporated into cassava starch film. The effect of MCC contents on morphology, appearances, thickness, mechanical properties, chemical interaction, surface wettability, moisture content, and water activity index of starch/MCC films were studied. The potential of UV-C disinfection for sterilizing these films for medical packaging was also investigated. Finally, the effects of UV-C sterilization of starch/MCC on film properties were also determined.

2. Materials and Methods

2.1. Materials

Cassava starch was sourced from Bangkok Inter Food Co., Ltd. (Bangkok, Thailand), and glycerol (≥99.5%) was acquired from Molecule Co., Ltd. (Bangkok, Thailand). Cassava pulp was supplied by Sanguan Wongse Industries Co., Ltd. (Nakhon Ratchasima, Thailand), and sodium hydroxide (NaOH, 98%) was provided by AGC Chemicals (Bangkok, Thailand) Co., Ltd. (Bangkok, Thailand). Acetic acid was obtained from RCI Labscan Co., Ltd. (Bangkok, Thailand), and sodium chlorite ($NaClO_2$, 80%) was sourced from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Deionized water was employed throughout the study.

2.2. Preparation of MCC

Cassava pulp with a mesh size of 150–250 µm was obtained from grinding and sieving. Cassava pulp was then dried in an oven at 120 °C for 24 h to eliminate excess moisture. About 200 g of dried cassava pulp was boiled in 4 L of alkali solution (4 wt% NaOH) at 80 °C under constant stirring for 2 h. Subsequently, deionized water was used to wash the alkali-treated cassava pulp until the pH level reached neutrality. The cassava pulp, after being treated with alkali, underwent drying at 80 °C for 24 h. An acetate buffer solution, comprising 27 g of NaOH and 75 mL of glacial acetic acid, diluted to 1 L of deionized water, was combined with 1 L of sodium chlorite solution containing 1.7% (w/v) $NaClO_2$ in water to create the bleaching solution. Following alkali treatment, the cassava pulp

was combined with the bleaching solution in a 1:20 ratio and stirred for 6 h at 80 °C. The bleached cassava pulp underwent rinsing with deionized water until the pH of the wash reached 7. Subsequently, the bleached cassava pulp was dried at 80 °C for 24 h and then ground and sieved with a mesh size of 38–63 µm. This processed sample was designated as MCC.

2.3. Characterization of MCC

2.3.1. Thermal Properties

Thermogravimetric analysis was performed utilizing the TGA/DSC 3+ STAR System (Mettler Toledo (Thailand), Bangkok, Thailand). The thermal decomposition temperature of each specimen was assessed under a nitrogen atmosphere at a flow rate of 20 mL/min. The analysis was conducted over a temperature range of 40–700 °C with a heating rate of 10 °C/min.

2.3.2. Structure and Chemical Composition

The structural and chemical composition of both cassava pulp and MCC were examined using a Tensor 27 FTIR spectrometer (Bruker Optics, Ettlingen, Germany). FTIR analysis was conducted within the spectral range of 4000–400 cm^{-1}.

2.3.3. Crystallinity

X-ray diffraction was employed to assess the crystallinity of both cassava pulp and MCC. The sample, in powdered form, was placed onto a sample holder and flattened to ensure uniform exposure to X-rays. The analysis was performed at room temperature using an X-ray diffractometer (D8-Advance Bruker AXS GmbH, Ettlingen, Germany) with a monochromatic CuK radiation source (λ = 0.1539 nm). The measurement was performed in step-scan mode using a 0.2° angle range from 5° to 40°, a step size of 0.02, and a scanning time of 0.5 min. The crystallinity index (CrI) was calculated using the Segal technique [26], as shown in Equation (1).

$$\text{CrI}\ (\%) = \frac{(I_{200} - I_{am})}{I_{200}} \times 100 \quad (1)$$

I_{200} is the maximum intensity of the (200) reflection at 2θ of 22°, and I_{am} is the lowest intensity of diffraction at 2θ of 18° for the amorphous region.

2.4. Preparation of Starch/MCC Films

The biocomposite films reinforced with MCC were prepared by a casting method [27]. Briefly, the cassava starch (5 g) was boiled in 50 mL of deionized water at 70 °C under constant stirring for 30 min, while MCC (0.05, 0.15, and 0.25 g) in 50 mL of deionized water were ultrasonicated at 25 °C for 30 min. Both cassava starch solution and MCC suspension were mixed at 70 °C under constant stirring for 30 min. Then, glycerol as plasticizer (1.5 g) was added and stirred at 70 °C for 3 h. The mixture solution was cast on the polystyrene plate and dried in the hot air oven at 60 °C for 24 h. All film samples were equilibrated in the desiccator at 25 °C, with a relative humidity of 50 ± 5% before characterization. The film formulations are shown in Table 1.

Table 1. The film formulation with various MCC contents.

Formulations	Cassava Starch (g)	Glycerol (g)	MCC (g)	Deionized Water (mL)
Starch	5	1.5	0	100
Starch/1%MCC	5	1.5	0.05	100
Starch/3%MCC	5	1.5	0.15	100
Starch/5%MCC	5	1.5	0.25	100

2.5. Characterization of Starch/MCC Films

2.5.1. Thickness

The film's thickness was measured with a digital thickness gauge. All tests showed the average results of six test specimens (n = 6).

2.5.2. Chemical Composition and Interactions

FTIR analysis was conducted to examine the chemical composition and interaction between starch and MCC in the composite films. This analysis utilized a Tensor 27 FTIR spectrometer (Bruker Optics, Ettlingen, Germany), with spectra recorded at room temperature within the spectral range of 4000–400 cm^{-1}.

2.5.3. Morphology

To examine the surface and cross-sectional morphology of the starch/MCC films, a field emission scanning electron microscope (FE-SEM, Carl Zeiss AURIGA®, Thuringia, Germany) was employed. The film specimens were coated with gold using a sputter coater. SEM images of the samples were captured at an acceleration voltage of 3 kV.

2.5.4. Mechanical Properties

A texture analyzer (TA.XT-PLUS, Texture Technologies Corp., London, UK) fitted with a 5 kg load cell was used to measure the starch/MCC films' Young's modulus, tensile strength, and elongation at break. Film samples were attached to the center of the tensile grasp jaw, measuring 50 mm in length and 10 mm in width. The grasp jaw kept the sample's upper and lower parts, each measuring 10 mm, firmly in place, providing a gauge length of 30 mm for the tensile test. Tensile testing was carried out at a speed of 10 mm/min. Each measurement is the average of five test specimens' values (n = 5).

2.5.5. Surface Wettability

To evaluate the surface wettability of the starch/MCC films, the water contact angle was determined. A precise volume of 15 µL of distilled water was deposited onto the surface of each sample. Following a 60 s exposure at room temperature, the image of the water droplet on the sample surface was captured using a USB digital microscope (1600×) and analyzed using ImageJ software version 1.53t (Wayne Rasband NIH, Washington, DC, USA). Three samples were analyzed per group. (n = 3).

2.5.6. Moisture Content

The moisture content of the starch/MCC films was analyzed utilizing a moisture analyzer (Sartorius MA 30, Sartorius Lab Instruments GmbH & Co. KG, Lower Saxony, Germany). Initially, the film was weighed to obtain its initial weight (W_i). Subsequently, the film was subjected to drying at 105 °C until its weight remained constant (W_d). The moisture content was then calculated using the following Equation (2). Each group comprised three samples (n = 3).

$$\text{Moisture content } (\%) = \frac{(W_i - W_d)}{W_i} \times 100 \quad (2)$$

2.5.7. Water Activity Index

The water activity index (aw) was analyzed using a water activity meter (AquaLab equipment, ICT International, Armidale, Australia). To conduct the determinations, the samples were kept under controlled conditions at 50% ± 5 relative humidity (RH) and a temperature of 25 ± 1 °C. The sample was placed in a cup, which was then positioned within the water activity meter. The sample chamber lid was sealed over it, and it was left to reach vapor equilibrium. An infrared beam focused on a tiny mirror determined the precise dew point temperature of the sample, which was then translated into water activity. Each group comprised three samples (n = 3).

2.6. UV Sterilization and Heat Sealing

UV sterilization and heat sealing were conducted in a laminar flow cabinet (Heal Force, Shanghai, China). Before undergoing the UV sterilization process, the starch/MCC film was positioned within an open sterile Petri dish. Each side of the film sample was exposed to UV-C radiation emitted by a lamp with a wavelength of 254 nm. The sterilization process times were 30 min for each side. The commercial sterilized gauze pad (5.08 × 5.08 cm^2 SOS® Plus, Yangzhou Super Union Import & Export Co., Ltd., Yizheng, China) was inserted between both sterilized rectangular films (7.62 × 7.62 cm^2). After that, the film was sealed on all four sides using a heat-sealing machine (Metronic Impulse Sealer, Jiaro international, Dali, China).

2.7. Sterility Tests

After the commercial sterilized gauze pad was stored in the film package at room temperature with a relative humidity of 50 ± 5% for 7 days, it was investigated for contamination of microorganisms resulting from contact with the starch/MCC film package. If there is no growth of microorganisms, the sterility of starch/MCC films passes the test. To assess the efficacy of the sterilization process, sterility tests were conducted in accordance with the guidelines outlined in the European Pharmacopoeia 6th Edition [20]. Soybean casein digest medium (SCDM) and fluid thioglycollate medium (FTM) were prepared for this purpose. The sterilized gauze was incubated in 20 mL SCDM at 25 °C and in 20 mL FTM at 37 °C for 7 days. Any growth of microorganisms was determined by the total plate count (TPC) method. In this study, the commercial product (sterilized gauze pad and sterilized sealing film, SOS® Plus, Yangzhou Super Union Import & Export Co., Ltd., Yizheng, China) was used as a control group. All tests were performed in triplicate (n = 3).

2.8. Statistical Analysis

General data were calculated using Microsoft Excel 2021 and reported as mean ± standard deviation (SD).

3. Results and Discussion

3.1. Characterization of MCC

The yield of MCC from the cassava pulp is calculated based on the amount of MCC obtained compared to the initial amount of cassava pulp used. In this study, the MCC yield is given as 14.92%. This is higher than the extraction of MCC from cassava pulp in previous research, MCC yield of 7.20% [25].

The FTIR spectra of cassava pulp and MCC are shown in Figure 1a. Both cassava pulp and MCC samples represented –OH stretching at peaks between 3600 and 3000 cm^{-1}. This band was associated with cellulose's intermolecular hydrogen bonding [28,29]. The FTIR peaks of cassava pulp and MCC at 898 cm^{-1}, 1204 cm^{-1}, and 1318 cm^{-1} were assigned as beta glycosidic linkages of the glucose ring, OH deformation, and CH_2 wag. These bands were related to the cellulose components's structure [30,31]. C=O stretching and C=C aromatic ring, which are related to lignin and hemicellulose, were represented by the peaks of cassava pulp at 1732 cm^{-1} and 1505 cm^{-1}, respectively [30,31]. These peaks clearly disappeared in the MCC, showing that hemicelluloses and lignin were partially removed from the fiber structure, resulting in increased cellulose purity.

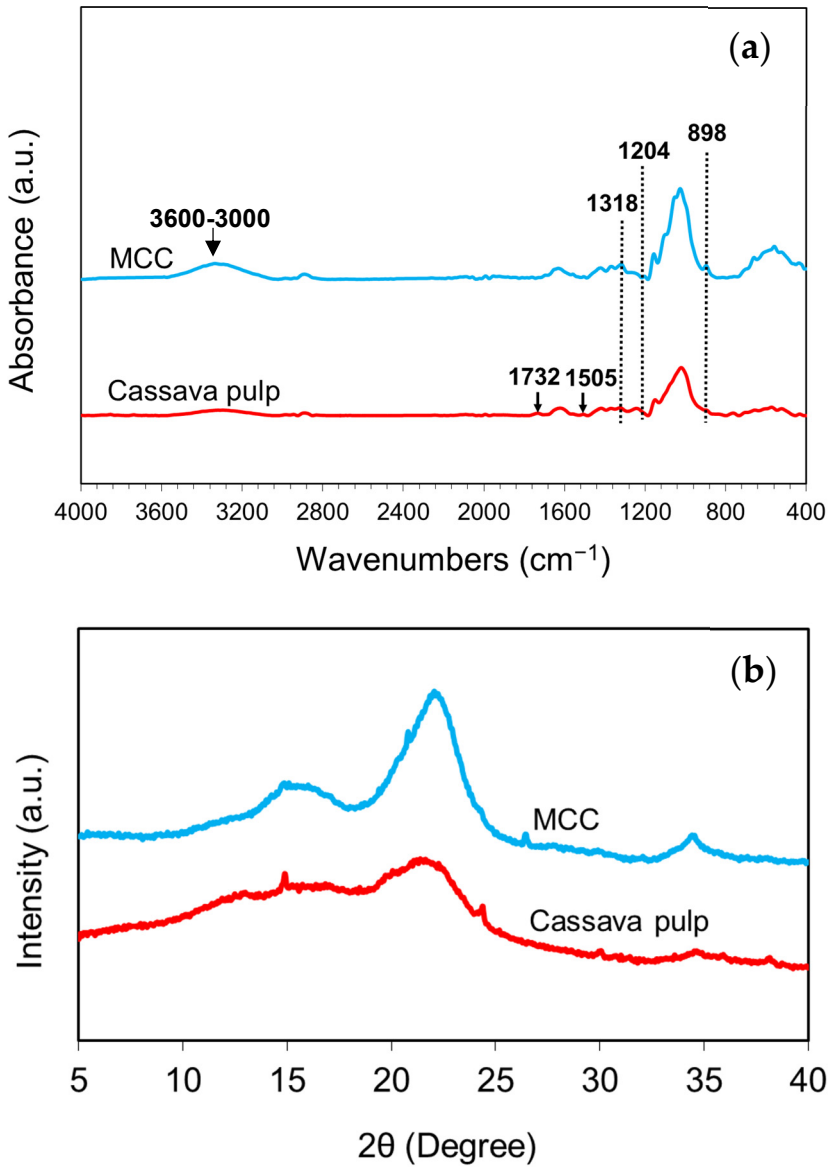


Figure 1. (**a**) FTIR spectra and (**b**) XRD spectra of cassava pulp and MCC.

In Figure 1b, the XRD curve illustrates distinct crystalline peaks at 2θ values of 16° and 22° for both cassava pulp and MCC. Notably, the crystalline peak of MCC displays a higher intensity compared to that of cassava pulp. The calculation of the crystallinity index (CrI) for all samples is depicted in Table 2. MCC demonstrates a notably high crystallinity of 47%, attributed to the removal of hemicelluloses and lignin bound to the cellulose fibers. Conversely, cassava pulp exhibits the lowest CrI at 19%.

Table 2. The crystallinity index (CrI) of cassava pulp and MCC.

Sample	Amorphous Region Peak Intensity (a.u.)	Crystalline Region Peak Intensity (a.u.)	CrI (%)
Cassava pulp	5441	6725	19.10
MCC	5070	9550	46.91

Thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG) are techniques used to study the thermal stability and decomposition behavior of materials. These curves provide insights into how a material's weight changes as it is heated over time,

and DTG gives the rate of weight loss at each temperature. The TGA and derivative thermogravimetry (DTG) curves of cassava pulp and MCC are shown in Figure 2a,b. Both cassava pulp and MCC experience weight loss below 100 °C due to moisture evaporation. This is typical for many organic materials, as they often contain some level of moisture that evaporates when heated. The maximum thermal decomposition temperature is the temperature at which the material experiences its highest rate of decomposition. For cassava pulp, this temperature is 312 °C, while for MCC, it is 343 °C. This indicates that MCC has higher thermal stability compared to cassava pulp because it can withstand higher temperatures before undergoing significant decomposition. The higher thermal stability of MCC compared to cassava pulp suggests that MCC is more resistant to thermal degradation. This could be attributed to differences in chemical composition. For example, MCC is predominantly composed of cellulose, which has a high thermal stability compared to other components found in cassava pulp, such as hemicellulose, lignin, pectin, starch, and lipid [32]. The mention of alkali treatment and bleaching implies that cassava pulp undergoes a chemical treatment process to remove certain components and improve its properties. The comparison between cassava pulp and MCC in terms of their thermal properties suggests that MCC is more thermally stable due to its higher cellulose content, while cassava pulp can be modified via chemical treatments to enhance its thermal stability by removing less stable components.

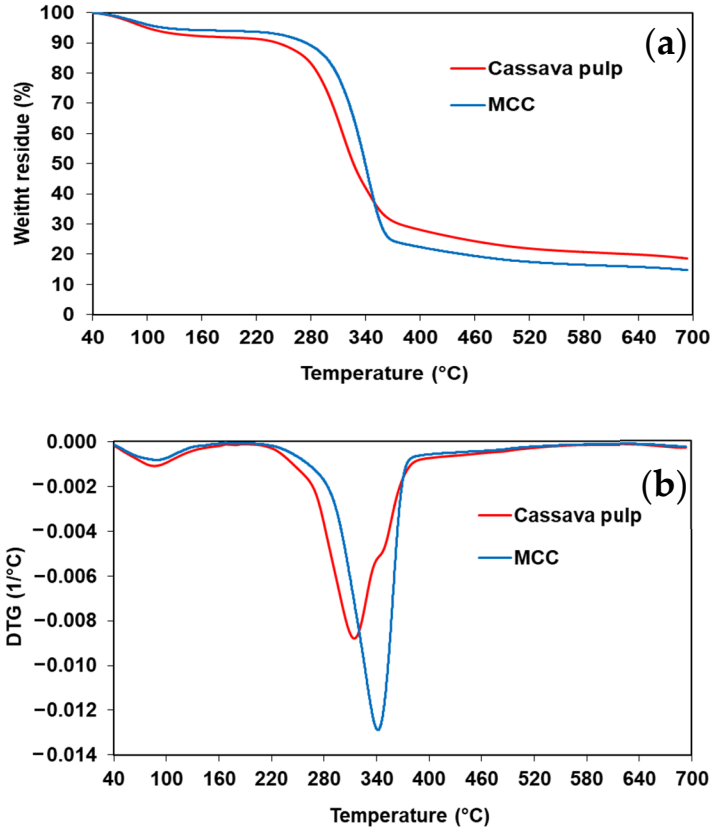


Figure 2. (**a**) TGA spectra and (**b**) DTG spectra of cassava pulp and MCC.

3.2. Characterization of Starch/MCC Films

This investigation involved the incorporation of MCC at varying contents (0, 1, 3, 5% *w*/*w* of starch) into cassava starch films. The study aimed to assess the influence of MCC content on the morphology, appearance, thickness, mechanical properties, chemical interaction, surface wettability, water activity index, and moisture content of the starch/MCC films.

3.2.1. Appearances and Thickness

Starch films incorporated with MCC at various contents (0%, 1%, 3%, and 5% *w*/*w* of starch) and glycerol as a plasticizer were successfully prepared, as shown in Figure 3a–d. Glycerol enhances film flexibility and prevents brittleness by improving their capacity to deform without fracturing [33]. The films are strong enough to be handled without deformation and are non-brittle, making them suitable for further investigation of physicochemical and mechanical properties.

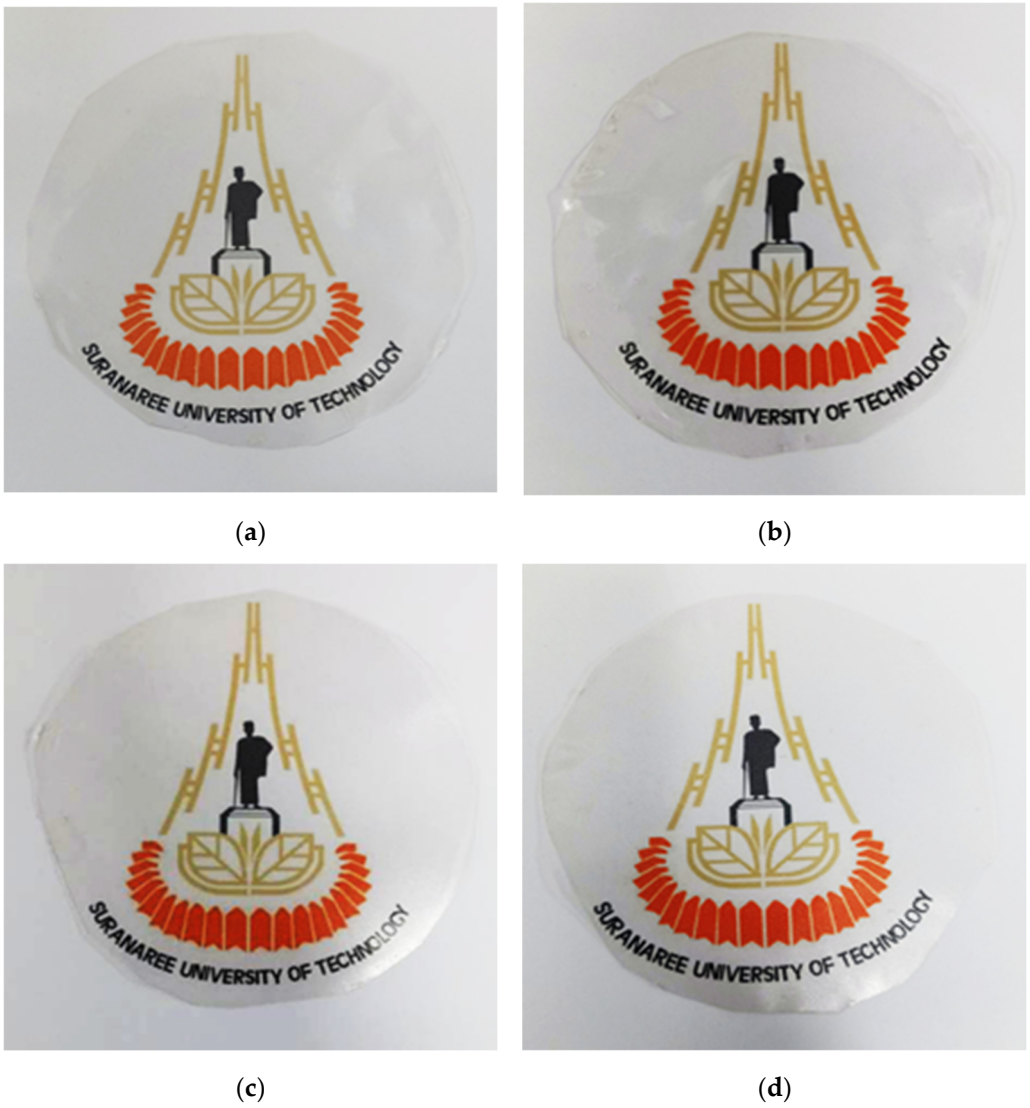


Figure 3. Appearances of films (**a**) Starch, (**b**) Starch/1%MCC, (**c**) Starch/3%MCC, and (**d**) Starch/5%MCC.

Transparency is an essential characteristic of packaging materials, as it allows consumers to visually inspect the product before purchasing. The study found that all prepared films were uniformly light-transparent, meaning that the logo and alphabet could be seen clearly through the films, as shown in Figure 3. This indicates that incorporating MCC into starch films within the range of 1% to 5% *w*/*w* of starch did not adversely affect the transparency of the resultant films. This suggests that the films have good transparency, which can enhance consumer experience and product visibility.

Typically, the thickness of commercial medical packaging films ranges from 25 to 750 μm, depending on their application [34]. This study focused on developing starch/MCC films for use in wound care packaging. The wound care packaging is mostly used in thickness ranges of 50–200 μm [35]. In our study, all prepared films showed thicknesses of 127–

144 (Table 3), indicating they were suitable for wound care packaging. The thickness of starch/MCC films slightly increased with increasing MCC contents in the films. The observed increase in thickness of starch/MCC films with rising MCC contents is in line with previous findings, such as those reported by Wahab et al. (2023) [36]. They found that the addition of 1% (*w*/*w*) MCC extracted from banana (Musa Saba) midrib residues increased the thickness of wheat starch films from 165 to 181 µm. As the concentration of MCC increases in the films, the overall solid content also increases. This leads to a higher volume of material being deposited, resulting in thicker films. Furthermore, the thickness can be adjusted for other medical packaging applications by controlling the solution volume for film casting. This flexibility allows for customization of the film thickness to meet the requirements of different packaging needs within the medical industry.

Table 3. Thickness of films (n = 6).

Formulations	Thickness (µm)
Starch	127 ± 8
Starch/1%MCC	127 ± 6
Starch/3%MCC	139 ± 6
Starch/5%MCC	144 ± 9

3.2.2. Chemical Composition and Interactions

Figure 4 illustrates the FTIR spectra of MCC, cassava starch film, and cassava starch films augmented with various contents of MCC (1%, 3%, and 5%). In the FTIR spectrum of MCC, peaks within the range of 3600–3000 cm^{-1} are attributed to the stretching of –OH. Additionally, the peaks observed at 1204 cm^{-1} and 1318 cm^{-1} are assigned to OH-deformation and CH_2 wag, respectively, representing structural components of cellulose [30,31]. The FTIR spectrum of the pure cassava starch film shows many absorption peaks. The stretching vibration of the O-H bond of intra- and intermolecular bonded -OH groups in starch is shown by the peak at about 3286 cm^{-1} [37]. The stretching vibration of the C-H bond in aliphatic methyl (-CH_3) or methylene (-CH_2) groups is indicated by the band at about 2922 cm^{-1}. Moreover, the carbonyl group's (C = O) vibrational stretching is responsible for the prominent signal at 1645 cm^{-1}. The findings resemble the research conducted by Debnath, B. et al. (2022) [11]. However, some studies have suggested that this sharp band may be due to the O-H bending of absorbed water molecules in the starch films [6]. The vibration of C-OH bending and -CH_2 bending in the plane are represented by peaks at 1414 cm^{-1} and 1318 cm^{-1}, respectively. The peak at 1150 cm^{-1} is attributed to the C-O-C ant symmetric bridge stretching, while the peaks at 1078 cm^{-1} and 997 cm^{-1} can be attributed to the C-O-H and C-O stretching vibration in the respective functional groups C-O-C [38]. The peak at 928 cm^{-1} represents asymmetric C-O stretching vibration from the glycosidic linkage, and the peak at 860 cm^{-1} indicates symmetrical CH and CH_2 deformation in starch [37]. The FTIR spectra of the pure cassava starch film and the MCC reinforced films exhibit similarities, indicating a similar chemical structure containing the same functional groups. Different amounts of MCC incorporated into the cassava starch-based films in the FTIR spectra do not demonstrate significant differences in functional groups. Notably, peaks at 1318 cm^{-1} and 1204 cm^{-1}, associated with distinct cellulose peaks, are evident in all starch/MCC films. Strong interactions between MCC and cassava starch molecules are confirmed by the remarkable drop in peak intensity at 3286 cm^{-1} corresponding to the -OH group with increasing MCC content [6]. The amount of -OH groups decreases as a result of this interaction, which causes polymer chains to lose hydroxyl groups in the form of water molecules [6].

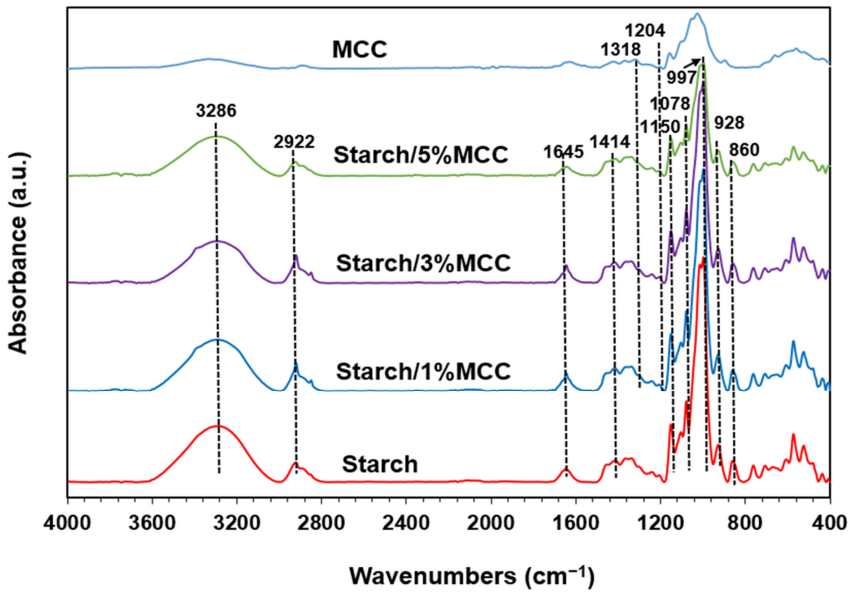


Figure 4. FTIR spectra of MCC, starch film, and starch/MCC films.

3.2.3. Morphology

The FE-SEM micrographs provided in Figure 5 illustrate the surface and cross-section characteristics of cassava starch films both with and without the inclusion of MCC. In Figure 5a, the micrograph depicts a smooth surface of the starch film, indicating a uniform composition without any apparent phase separation. This suggests that starch film alone exhibits homogeneous properties. Figure 5b–d depict starch films with varying contents of MCC (1%MCC, 3%MCC, and 5%MCC); there is visible evidence of MCC distribution on the surface of the films. This indicates that MCC is effectively incorporated into the starch matrix and is evenly dispersed across the surface. Moreover, the cross-section images reveal good adhesion between MCC and the starch matrix, with no discernible phase disengagement because of the strong interfacial bonding between MCC and the starch matrix, as confirmed by FTIR results.

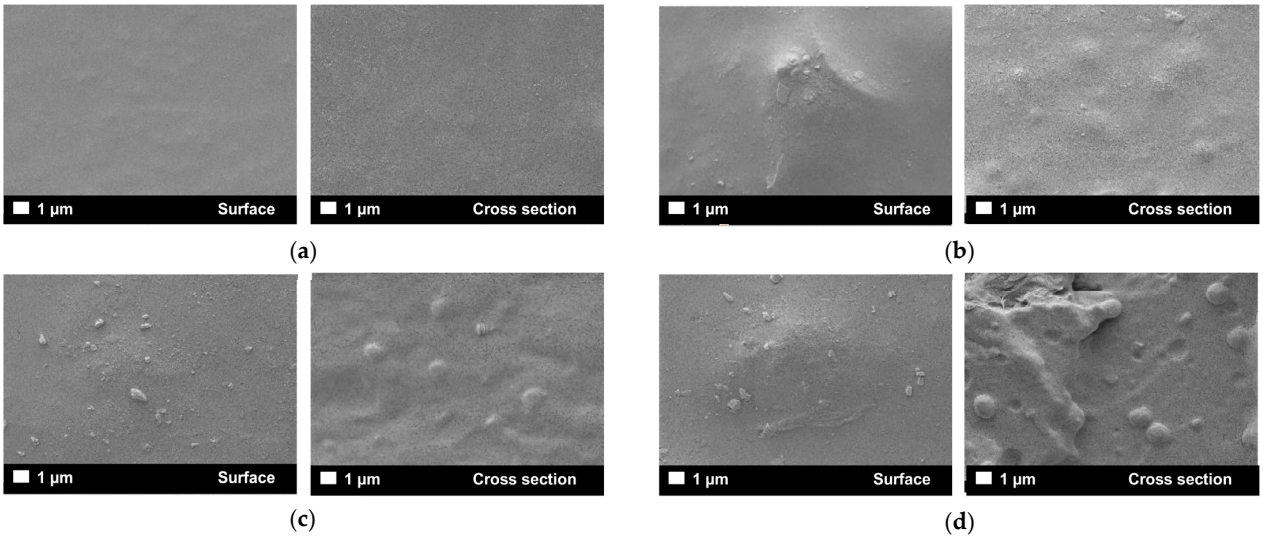


Figure 5. FE-SEM micrographs of surface and cross-section of films (**a**) Starch, (**b**) Starch/1%MCC, (**c**) Starch/3%MCC, and (**d**) Starch/5%MCC.

3.2.4. Mechanical Properties

The mechanical characteristics of packaging films, especially those intended for medical use, are crucial for ensuring their ability to withstand damage and maintain integrity during handling and storage. In this study, the tensile stress–strain curves of cassava starch films with various contents of MCC are analyzed to evaluate their mechanical properties, as shown in Figure 6a. The stress–strain behavior of starch films, as well as those with 1% and 3% MCC contents, indicates soft and flexible materials. These films exhibited elongation at a break of more than 30%, indicating their ability to deform under stress without fracturing abruptly. However, the starch films with 5% MCC exhibited brittle behavior, with less than 5% elongation at break. A material with low elongation at break, such as less than 5%, is typically considered brittle [39]. This suggests that the higher concentration of MCC makes the films more rigid and less able to undergo plastic deformation before fracture. To assess the effects of MCC content on mechanical properties, the tensile strength, Young's modulus, and elongation at break are illustrated in Figure 6b–d and summarized in Table 4. The starch film showed the highest elongation at break (89.94%) while their tensile properties exhibited the lowest, which showed a tensile strength of 2.85 MPa and a Young's modulus of 83.85 MPa. As MCC concentration increased from 1% to 5% (w/w of starch), Young's modulus and tensile strength of the films increased from 112.12 MPa to 488.89 MPa and 3.21 MPa to 11.18 MPa, respectively, whereas their elongation at break decreased from 44.74% to 4.15%. This is because MCC, being a reinforcing filler, enhances the mechanical properties of the films by providing structural support [11]. The enhanced strength can be attributed to the effective dispersion and favorable compatibility between the MCC filler and cassava starch matrix, facilitated by the formation of a robust H-bonded network, as corroborated by findings from FTIR and FE-SEM analyses. Conversely, the elongation at break diminishes with increasing MCC concentration. This is an anticipated outcome as higher MCC content renders the films stiffer, reducing their ability to stretch before fracturing. This finding aligns with previous literature [11,40]. Currently, commercial biodegradable packaging films include hybrid films made of nondegradable synthetic polymer and biodegradable synthetic polymer (such as co-polyester/polylactic acid, BIO-FLEX® F 6611), as well as packaging films made from a combination of natural and biodegradable synthetic polymers (such as starch/polybutylene adipate terephthalate blend film). The commercial biodegradable packaging films with different types of polymers had different film characteristics. The commercial starch/polybutylene adipate terephthalate exhibited a tensile strength of 5.6 MPa and high flexibility with elongation at a break of up to 137%. On the other hand, the commercial co-polyester/polylactic acid showed low flexibility with elongation at a break of 9.7% but exhibited excellent stiffness with high Young's modulus and tensile strength, as shown in Table 4 [41,42]. Unfortunately, starch films and starch/1% MCC exhibited low mechanical strength, while the starch film with 5% MCC displayed brittle behavior, resulting in mechanical properties inferior to those of both commercial biodegradable packaging films. The starch film with 3% MCC, despite exhibiting less flexibility compared to commercial starch/polybutylene adipate terephthalate, still possesses sufficient elasticity, as evidenced by an elongation at a break of 32.54%. This implies that they can stretch to a certain extent before breaking, a critical quality for packaging materials. Additionally, these films demonstrate enough strength to be effectively used as film packaging, and in fact, they even surpass the strength of commercial starch/polybutylene adipate terephthalate film. Moreover, the starch/3% MCC film displayed greater flexibility than commercial co-polyester/polylactic acid film. However, they exhibited lower mechanical strength compared to commercial co-polyester/polylactic acid film. Importantly, the mechanical properties of the starch films with 3% MCC fall within the range observed for both commercial packaging films. These findings suggest that the starch/3%MCC film holds promise as a viable option for biodegradable packaging solutions, offering a balance of elasticity, strength, and flexibility.

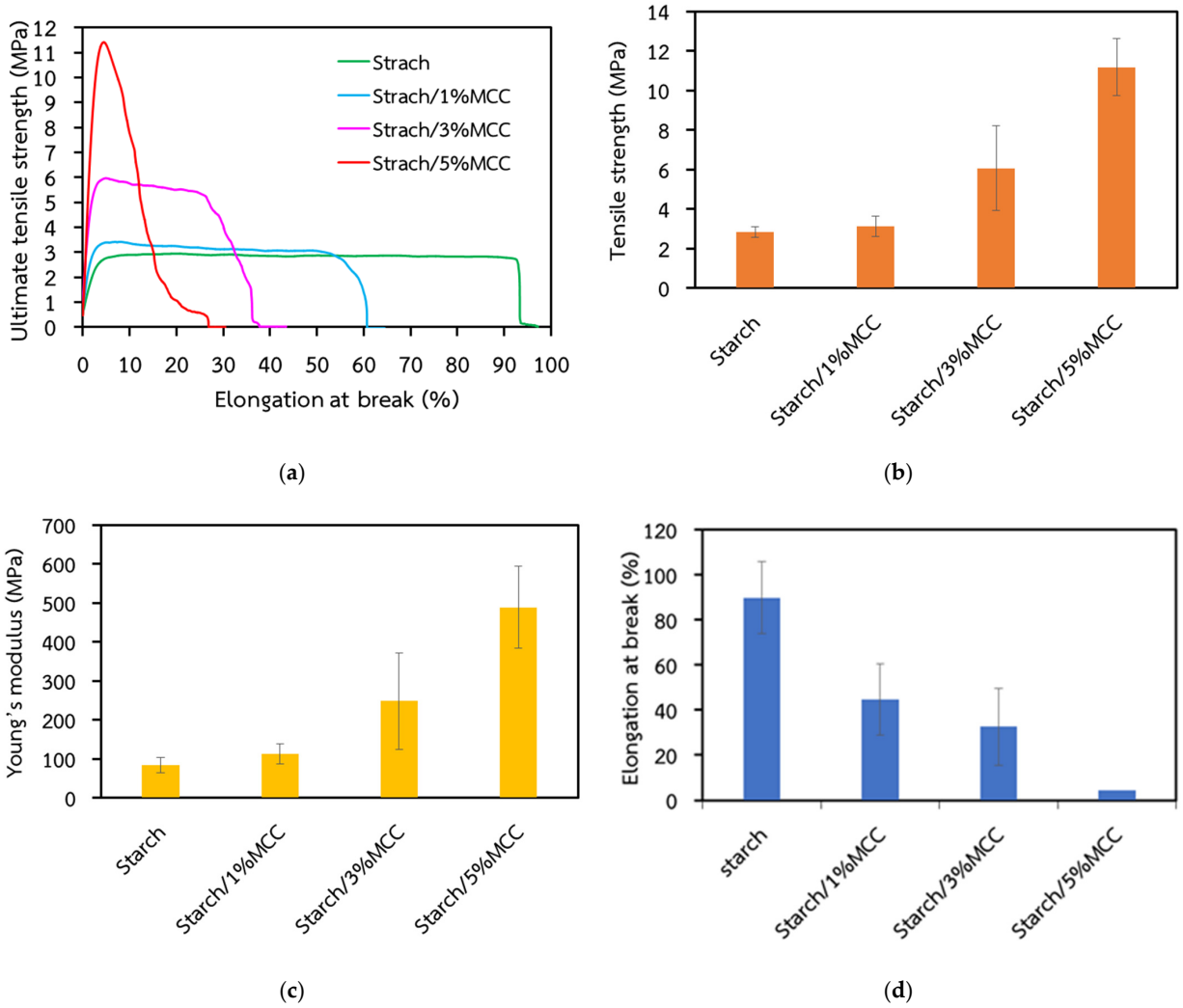


Figure 6. Mechanical properties, (**a**) tensile stress–strain curve, (**b**) tensile strength, (**c**) young's modulus, and (**d**) elongation at break of films (n = 5).

Table 4. The comparison of different starch film materials in mechanical properties (n = 5).

Film Formulations	Young's Modulus (MPa)	Tensile Strength (MPa)	Elongation at Break (%)	References
Starch	83.85 ± 18.9	2.85 ± 0.27	89.94 ± 16.00	This work
Starch/1%MCC	112.12 ± 25.8	3.21 ± 0.52	44.74 ± 15.89	
Starch/3%MCC	248.63 ± 123.3	6.06 ± 2.13	32.54 ± 17.23	
Starch/5%MCC	488.89 ± 105.6	11.18 ± 1.46	4.15 ± 0.18	
Commercial starch/polybutylene adipate terephthalate	/	5.6	137	[41]
Commercial co-polyester/polylactic acid (BIO-FLEX® F 6611)	2730	16	9.7	[42]
Cassava starch/ bamboo cellulose nanocrystals	/	4.20	10.30	[6]
Corn starch/ bamboo cellulose nanofiber	216.09 ± 34.86	5.07 ± 0.15	22.23 ± 8.02	[41]
Maize starch	1.82 ± 6.70	1.49 ± 0.26	51 ± 6	[43]
Oat starch	1.80 ± 0.90	0.36 ± 0.51	27 ± 5	[43]
Corn starch/MCC derived from elephant grass	162.14 ± 6.0	6.23 ± 0.51	24.36 ± 1.7	[11]

Note: / means not mentioned.

This indicates that the addition of MCC at this concentration effectively improves the mechanical performance of the films, potentially making them suitable for medical packaging applications. Furthermore, numerous investigations have reported the mechanical properties of various starch film materials; however, their mechanical characteristics were less than the starch/3%MCC film (Table 4). This suggests that the incorporation of MCC enhances the mechanical properties beyond what can be achieved with starch alone or with lower contents of MCC.

3.2.5. Surface Wettability

The surface wettability of packaging films plays a crucial role in maintaining the integrity of the packaged product. Excessive wetting, especially in highly wettable or hydrophilic films, can lead to liquid infiltration, potentially causing damage to the enclosed product. Therefore, it is imperative for packaging films to exhibit low wettability or high hydrophobicity. Wettability, quantified by the water contact angle, serves as a key metric to assess a material's surface hydrophilicity or hydrophobicity [44]. A surface is deemed hydrophobic when the water contact angle falls within the range of 150° to 90°. If the water contact angle surpasses 150°, the surface is classified as superhydrophobic. Conversely, a surface is labeled hydrophilic when the water contact angle ranges between 90° and 10°. Surfaces with water contact angles below 10° are considered superhydrophilic. Figure 7 depicts the water contact angle of cassava starch films with and without MCC. Initially, the water contact angle of the starch films is measured at 69.17°, indicating the hydrophilic nature of the film surface. Upon the addition of MCC into the films, up to 3% (w/w of starch), a noticeable increase in the water contact angle is observed, reaching 102.82°. It is noteworthy that there is no significant difference in the water contact angle between the starch/3%MCC and starch/5%MCC films. This suggests that the presence of MCC substantially enhances the hydrophobicity of the film surface. The FTIR spectra depicted in Figure 4 validate the strong interaction between MCC and cassava starch via hydrogen bonds, leading to the formation of a network among the polymer chains of starch. This interaction effectively diminishes the affinity between water and the film surface, thereby inhibiting water penetration [11]. Additionally, MCC diminishes the free space between the molecules of cassava starch films, thereby restricting water absorption [45]. Chen et al. (2020) and Debnath et al. (2022) have also reported that the incorporation of MCC can decrease the hydrophilicity of biocomposite films [11,46]. The findings of the present study suggest that MCC could serve as a promising filler to enhance the hydrophobicity of cassava starch films for packaging applications.

The investigation of water dynamic contact angle on films revealed intriguing findings as shown in Figure 8. Initially, all films displayed a decline in water contact angle from 1 to 60 min of exposure. Specifically, starch films and starch/1%MCC exhibited a contact angle below 90° after 10 min of water contact, indicative of increased water penetration and swelling at 60 min. Starch/3%MCC and starch/5%MCC initially demonstrated contact angle exceeding 90° after 10 min of water exposure. However, over time, their contact angle decreased, falling below 90° between 10 and 60 min. This observation suggested that starch/3%MCC and starch/5%MCC initially inhibited water penetration into the film surfaces within the first 10 min. Nonetheless, as the water contact time surpassed 10 min, the hydrophobicity of the films diminished, facilitating water penetration. The observation of starch/3%MCC and starch/5%MCC initially exhibited a contact angle above 90° after 10 min of water exposure, implying a temporary resistance to water penetration, attributed to the enhanced hydrophobicity conferred by MCC. This finding underscores the potential of MCC to modify the surface properties of starch films, rendering them less susceptible to immediate water infiltration. However, the subsequent reduction in contact angles beyond 10 min signifies a transition towards increased water permeability, indicating a compromise in the hydrophobic barrier provided by MCC. This phenomenon could be attributed to factors such as hydration-induced swelling or structural rearrangement within the film matrix, ultimately leading to the loss of hydrophobic character and enhanced water uptake.

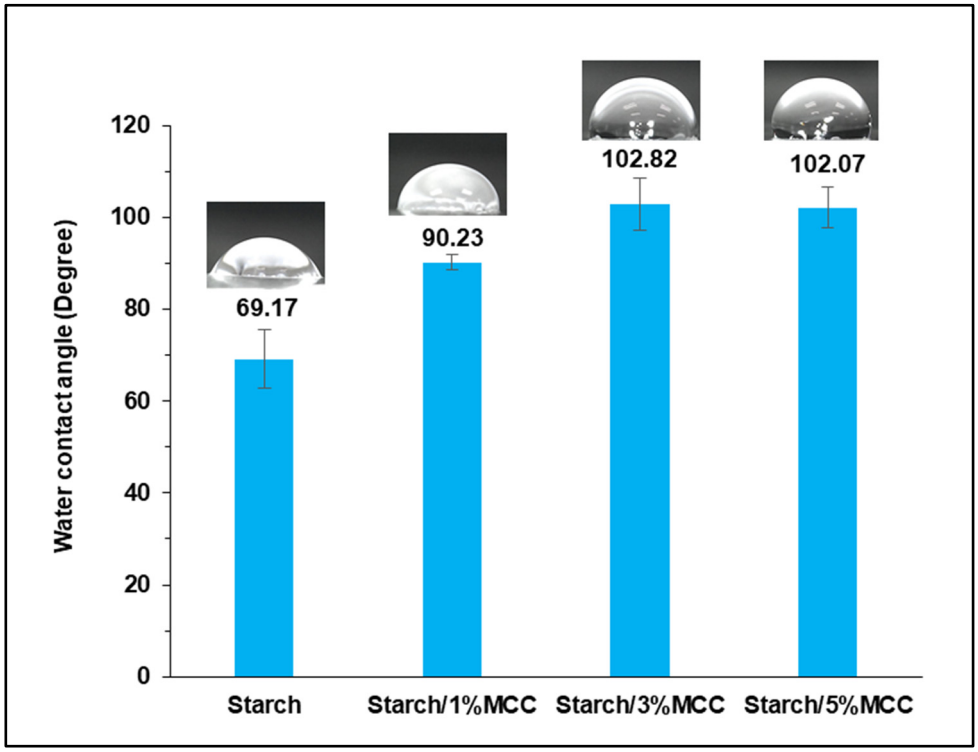


Figure 7. Water contact angle of films (n = 3).

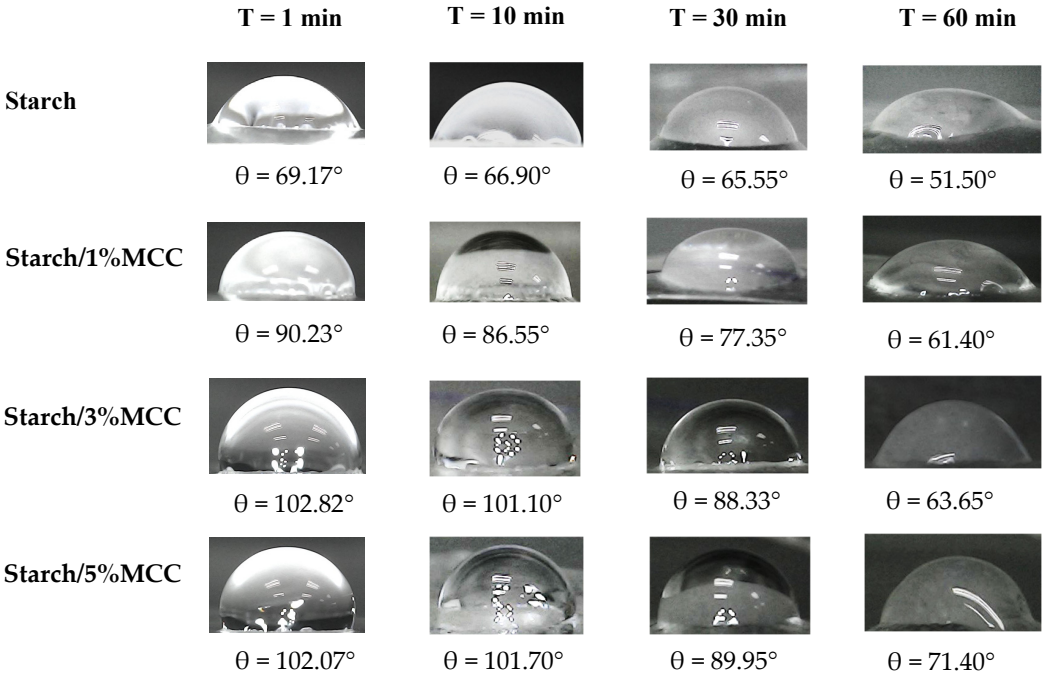


Figure 8. Water contact angle images droplets on the surface of the cassava starch/MCC films with times.

3.2.6. Moisture Content and Water Activity Index

The moisture content and water activity levels in packaging play a vital role in preserving the safety, stability, and quality of packaged products. By controlling these parameters, manufacturers can mitigate the risk of microbial contamination and chemical degradation, ensuring consumer safety and satisfaction.

The moisture content of the starch film exhibited a slight decrease from 14.20% to 12.98% upon the incorporation of 5% (*w*/*w*) MCC (Table 5). Desire et al. (2021) reported a similar trend in their study [47]. They found that the addition of 7% (*w*/*w*) MCC slightly decreased the moisture content of the cassava starch film from 14.24% to 13.55%. This reduction in moisture content can be attributed to the formation of more intermolecular hydrogen bonds between MCC and the hydroxyl groups of starch. Consequently, the availability of free -OH groups decreases, leading to a reduction in the interaction between water and the starch-OH groups. Consequently, the moisture content of the films tends to decrease [48].

Table 5. Moisture content and water activity index of films (n = 3).

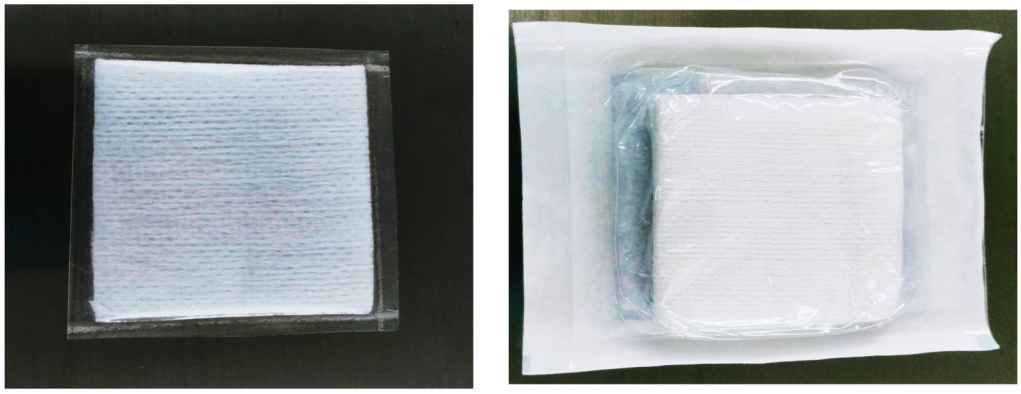
Formulation	Moisture Content (%)	Water Activity Index
Starch	14.20 ± 1.42	0.50 ± 0.003
Starch/1%MCC	13.76 ± 1.55	0.48 ± 0.004
Starch/3%MCC	14.07 ± 1.05	0.51 ± 0.004
Starch/5%MCC	12.98 ± 0.51	0.54 ± 0.004

Water activity quantifies the amount of free moisture in a product and is defined as the ratio of the vapor pressure of water in a material to the vapor pressure of pure water at the same temperature [49]. A water activity index below 0.6 indicates that the environment is relatively low in available water, which can be inhibitory towards the growth of microorganisms such as *S. aureus*, *E. coli*, and other coliforms [50]. From the results, as shown in Table 5, all starch films have water activity values lower than 0.6, ranging from 0.48 to 0.54, indicating that molds, yeasts, and bacteria are unable to grow and proliferate, ensuring safety for use.

The findings from all the results suggest that starch/3%MCC holds potential as a biocomposite film for medical packaging applications. It offers benefits such as excellent transparency, favorable mechanical properties, and surface hydrophobicity. Consequently, the starch/3%MCC film was chosen for further investigation in sterility determination and the impact of UV sterilization on its properties.

3.3. Sterility

The starch/3%MCC film was securely sealed on all four sides utilizing a heat-sealing machine, demonstrating excellent sealing integrity, as shown in Figure 9a. After storing the starch/3%MCC sealing film at room temperature for 7 days, the sterilized gauze pad was incubated in SCDM at 25 °C and FTM at 37 °C for 7 days and then investigated any growth of microorganisms by the TPC method. In this study, the commercial product (sterilized gauze pad and sterilized sealing film) was used in the control group (Figure 9b). In the sterility tests, it was found that there was no microbial growth after incubation in both the SCDM and the FTM at the 7 days with the gauze pad samples in the sterilized starch/3%MCC and commercial sealing film (Figure 10). The absence of microbial growth in the sterilized gauze pad with sealing in the sterilized starch/MCC film confirms that the UV-C sterilization, 30 min for each side at 254 nm, effectively eliminated any microorganisms present on the starch/MCC film in the thickness of 139 ± 6 µm, ensuring their sterility. This reinforces the effectiveness of the sterilization process in maintaining the sterility of the medical packaging, which is crucial for ensuring their safety and efficacy in medical applications.



(**a**) (**b**)

Figure 9. The appearance of the gauze pad packaging: (**a**) Starch/3%MCC film and (**b**) commercial film.

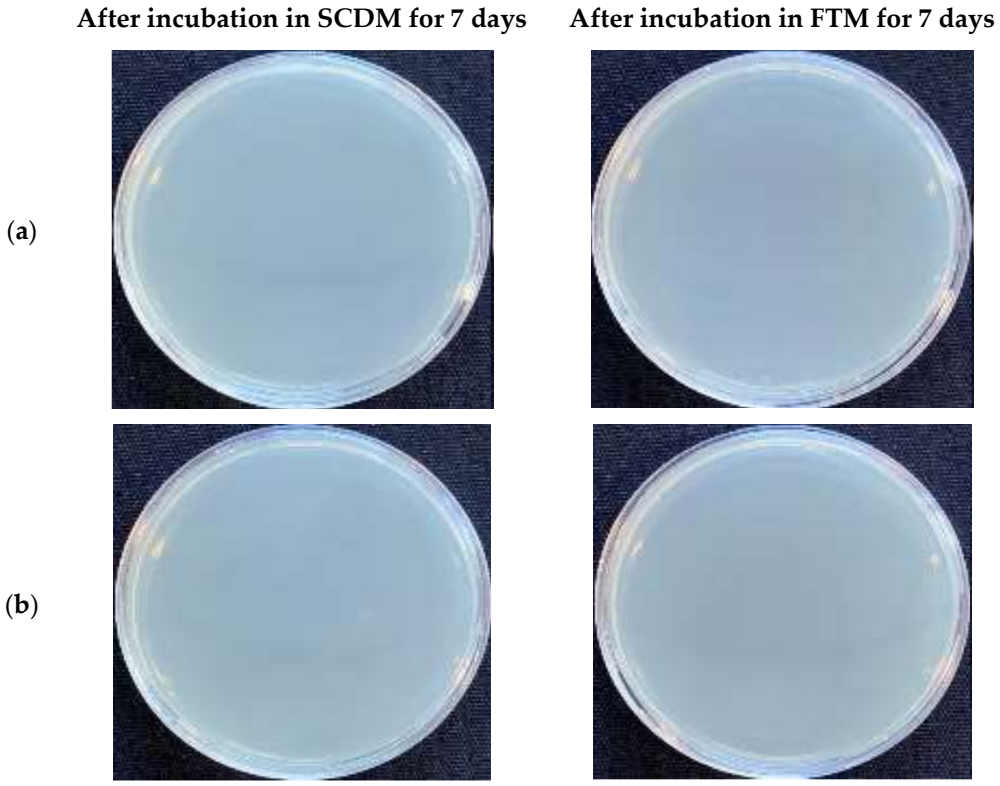
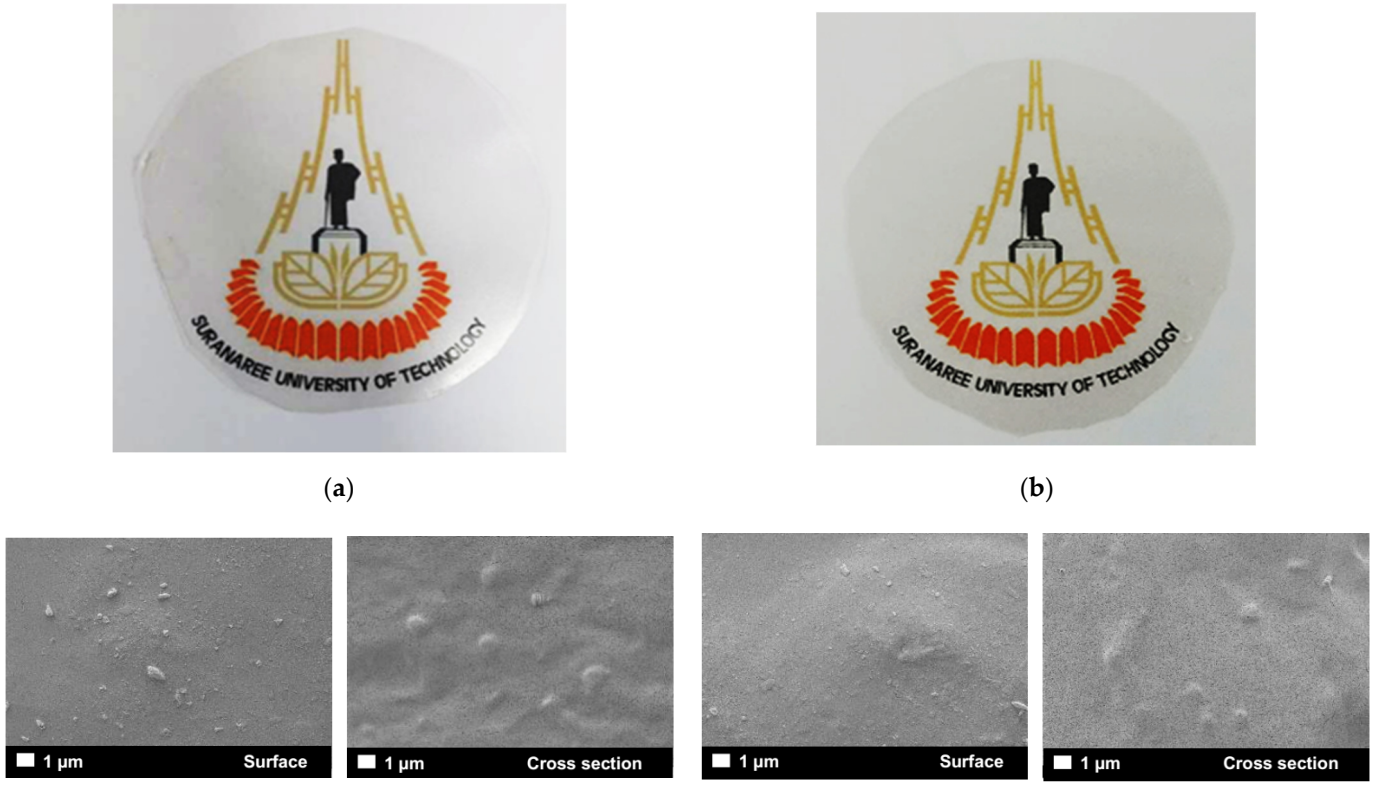


Figure 10. Total bacteria detected in (**a**) the gauze pad in sterilized starch/3%MCC sealing film after incubation in SCDM and FTM for 7 days and (**b**) the gauze pad in commercial sealing film after incubation in SCDM and FTM for 7 days.

3.4. Effect of UV Sterilization on Starch/MCC Film Properties

UV-C sterilization can potentially affect the mechanical properties of film packaging materials, depending on various factors such as the type of material, UV exposure duration, and intensity [20,51]. The prolonged exposure to UV light caused mechanical loss of film product. UV radiation is caused by breaking of the molecular chains of materials. In our study, the UV-C sterilization, 30 min for each side at 254 nm, did not affect the appearance, thickness, morphology, mechanical, surface wettability, moisture content, and water activity index of starch/3%MCC film (Figure 11 and Table 6). These results confirmed that applying UV disinfection is an alternative method for sterilization of transparent film packaging products.



(**a**) (**b**)

(**c**) (**d**)

Figure 11. Appearances of films. (**a**) Non-sterile Starch/3%MCC, (**b**) sterile Starch/3%MCC. FE-SEM micrographs of surface and cross-section of films. (**c**) Non-sterile Starch/3%MCC and (**d**) sterile Starch/3%MCC.

Table 6. The physicochemical properties of films with and without UV sterilization.

Film Formulations	Thickness (µm)	Young's Modulus (MPa)	Tensile Strength (MPa)	Elongation at Break (%)	Moisture Content (%)	Water Activity	Contact Angle (°)
Non-sterile Starch/3%MCC	139 ± 6	248.63 ± 123.49	6.06 ± 2.13	32.54 ± 17.23	14.07 ± 1.05	0.51 ± 0.004	102.82
Sterile Starch/3%MCC	138 ± 5	250.88 ± 80.93	6.17 ± 3.02	32.74 ± 11.93	14.23 ± 1.27	0.51 ± 0.002	101.45

4. Conclusions

Our study demonstrates the feasibility of developing biocomposite films utilizing cassava starch and MCC derived from cassava pulp for medical packaging applications. By incorporating MCC into cassava starch films at varying contents and employing glycerol as a plasticizer, films with desirable properties were successfully produced. The addition of MCC significantly enhanced the mechanical properties of the films, leading to substantial increases in tensile strength and Young's modulus while maintaining acceptable levels of transparency. However, it was observed that as the MCC content increased, the elongation at break decreased, suggesting a trade-off between strength and flexibility. Moreover, the introduction of MCC contributed to the reduction in film surface wettability, thereby enhancing surface hydrophobicity, which is advantageous for medical packaging applications where water-repellent is crucial. The starch/3%MCC holds promise as a biocomposite film for medical packaging applications, offering advantages in terms of good transparency, mechanical properties, and surface hydrophobicity. Importantly, our study validated the effectiveness of UV-C sterilization in ensuring the sterility of the starch/MCC films without

compromising their structural integrity. The absence of microbial growth in the sterilized films, as demonstrated by the sealed gauze pad experiment, underscores the reliability of this sterilization method for medical packaging applications.

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